SCIENTIFIC NOTE

Effect of handling and morbidity induction on weight, recovery, and survival of the Pacific Coast wireworm, *Limonius canus* (Coleoptera: Elateridae)

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Wireworms can recover from prolonged morbidity induced by exposure to insecticides (Vernon *et al.* 2008), but it is unclear to what extent wireworm physiology and survival are affected by repeated handling or morbidity inductions, which reduces larval weight and retards development (Nicolas and Sillans 1989) in some insects. It may be that differences in weights or survival in wireworms repeatedly made moribund, similarly handled but not made moribund, or not subjected to handling or morbidity might occur.

Larvae of the Pacific Coast wireworm, *Limonius canus* LeConte, become temporarily moribund after contact with tefluthrin-treated wheat seeds, and recover more quickly when re-exposed (van Herk and Vernon 2007), but it is not known if continued re-exposure further decreases recovery time. Wireworms repeatedly contact insecticide-treated seeds in the soil, thus a continued decrease in the morbidity duration may affect the insecticide's efficacy in the field. Here we discuss if wireworms continue to recover more quickly from tefluthrin-induced morbidity, and if this or handling affects their weight and the time to complete the larval instar.

Wireworms were collected from an organic farm in Kelowna, BC, in June 2007 and stored in soil at 15 °C. Late-instar, feeding wireworms were randomly allocated to one of three treatments (24–50 per treatment), the 'morbidity', 'handled', and 'control' treatments. All observations were made at 21±1 °C. In the 'morbidity' treatment, morbidity was induced, and wireworm health assessed following van Herk and Vernon (2007). Individual wireworms were placed for 2 min in 1.5-ml Eppendorf microcentrifuge tubes (Fisher Scientific) with a single, ungerminated wheat seed (cv. Superb) treated with Tefluthrin 20CS (20% tefluthrin w/v) at 10 g AI/100 kg seed and the fungicide Dividend XLRTA (3.21% difenoconazole, 0.27% mefenoxam) at 13 g AI/100 kg seed. Seeds were treated in 2007 by Syngenta Crop Protection Canada Inc. (Portage la Prairie, MB). After exposure, larvae were placed in individual Petri dishes lined with moistened filter paper to observe the onset of morbidity, and subsequently placed in separate, identical film canisters filled with finely screened soil with 20% moisture by weight. Wireworm 'health' was assessed every 10 min until 30 min after no further symptoms of morbidity were observed (i.e. 7-15 times). Morbidity was induced using this method at 24-h intervals for 4 consecutive days.

Wireworms in the 'handled' treatment were placed for 2 min in Eppendorf tubes with a single untreated wheat seed and, as above, observed for 20 min in a Petri dish, and then transferred to film canisters filled with soil. To expose these wireworms to handling comparable to those in the 'morbidity' treatment, the health of 'handled' wireworms was assessed at 10-min intervals 12 times on the first day and 10 times on the subsequent 3 d. Wireworms in the 'control' treatment were placed in film canisters and weighed, but were not placed in Eppendorf tubes or Petri dishes. Larvae were weighed individually for 5 consecutive d, approx. 6 h before wireworms in the 'handled' or 'moribund' treat-

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ments were placed in Eppendorf tubes the first 4 d, and at the same time of day on day five. Larvae were weighed for final time approx. 2 mo later, at which time the proportion moulted was recorded.

Wireworm recovery durations were compared among treatments with ANOVA, and the % decrease in recovery time between day 1 and days 2, 3 and 4 was calculated. Weight loss over time was compared among treatments with repeated measures ANOVA; orthogonal contrasts were used to compare wireworm weight loss between 'control' and 'handled', and between 'handled' and 'moribund' treatments. Proportions of larvae that moulted were compared with Chi-square analysis.

In the morbidity treatment, the time to recovery on day 1 was 88.8 ± 2.3 (SEM) min, similar to the 86.0 to 87.8 min reported by van Herk and Vernon (2007). Time to recovery on day 1 was significantly longer than on days 2, 3 and 4 (72.4 ± 1.8, 69.4 ± 1.8, 71.8 ± 1.8 min, respectively; F=20.70, df=3,196, P<0.0001), but recovery durations on the latter 3 d were not different (P>0.05). Recoveries on days 2, 3 and 4 were 18.5 – 21.8% more rapid than the initial recovery time. These data suggest that repeated contact with tefluthrin-treated seeds in the field will not likely make wireworms insensitive to the insecticide.

Analysis of individual wireworm weights over time using the first five measurements indicated a weight loss over time (F=8.35, df=4,384, P<0.0001, Table 1) and an interaction between weight loss and treatment (F=2.25, df=8, 384, P=0.02). These remained significant (P<0.05) when all six weighing days were included in analyses; orthogonal contrasts indicated greater weight loss in 'handled' than in 'control' treatments (F=3.04, df=5,480, P=0.01), but not between 'moribund' and 'handled' treatments (F=0.80, df=5,480, P=0.55). This suggests that weight loss in the 'moribund' treatment was due to handling alone, and that extensive handling of wireworms may cause stress or damage, perhaps including elevation of hemolymph sugar or lipids (Woodring *et al.* 1989). It is unlikely the observed weight loss was from desiccation as it continued from days 5 to 68 when wireworms were continuously in moist soil. Thus care must be taken to minimize handling events and/or trauma.

All wireworms were alive on day 68 with no difference in moulting in the treatments by day 68 (0.62, 0.52, 0.42, respectively; χ^2 = 2.79, df=2, P=0.25), so that repeated morbidity induction and handling did not affect survival or development.

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Table 1.
Mean (SEM) weight (mg) of *Limonius canus* larvae subjected to one of three treatments: 'Moribund': repeated handling plus morbidity induced by insecticide exposure; 'Handled': repeated handling only; 'Control': no handling or morbidity induction.

Treatment	N	day 1	day 2	day 3	day 4	day 5	day 68
Control	24	25.5 (1.2)	25.6 (1.2)	25.5 (1.2)	25.5 (1.2)	25.5 (1.2)	25.7 (1.2)
Handled	25	26.0 (1.1)	25.8 (1.1)	25.8 (1.1)	25.7 (1.1)	25.6 (1.1)	24.9 (1.2)
Moribund	50	25.4 (0.8)	25.5 (0.8)	25.1 (0.8)	25.1 (0.8)	25.1 (0.8)	24.7 (0.8)

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